encoding a polypeptide whereby said protein is expressed specifically in mammary cells of said transgenic mammal and said protein comprises a signal peptide, said peptide being effective in directing the secretion of said protein into the milk of said mammal, and wherein said transgenic mammal is selected from the group consisting of mice, rats, rabbits, pigs, sheep, goats and cows.

Please add the following new claim:

--16. An isolated DNA molecule capable of stimulating the expression of a heterologous gene, wherein said DNA molecule consists of the 5' 4.2 kb *Sau*3A - *Kpn*1 promoter of the mouse whey acidic protein gene.--

REMARKS

Claims 5, 10, 13 and 15 have been canceled and claim 16 has been added by amendment. Claims 1-4, 6-9, 11, 12, 14 and 16 are pending in the application. Applicants have amended the claims to more clearly define what the applicants consider to be the invention. Support for the language "wherein said transgenic mammal is selected from the group consisting of mice, rats, rabbits, pigs, sheep, goats and cows" in amended claims 1, 6, 11, 12 and 14 can be found at least on page 16, lines 19-20, of the specification. Support for new claim 16 can be found at least in Example 11 of the specification. For the examiner's convenience, applicants attach a copy of the pending claims to this response as Exhibit 1. No new matter has been added by way of these amendments.

Applicants request the examiner to remove the finality of the pending office action under 37 CFR §1.129(a). A request and the required fee accompany this response.

In the office action dated July 27, 1995, the examiner made one objection and three rejections. In response, applicants respectfully submit the following remarks.

I. Objection Under 35 USC §112, First Paragraph

The examiner has objected to the specification under the first paragraph of §112 on the basis that the specification does not provide support for the presently claimed invention. In particular, the examiner has stated that applicants must deposit DNA molecules that contain the mouse whey acidic protein (WAP) promoter and the human protein C gene. Applicants respectfully traverse this basis for objection.

Applicants respectfully note that they have demonstrated that one of skill in the art could have obtained the WAP promoter and protein C DNA fragments from starting materials, nucleotide sequences, and amino acid sequences that were available as of the filing date of the present application. See pages 8-13 of the response filed on January 24, 1994. Also see pages 1-9 of the response filed on September 14, 1994. Applicants respectfully reiterate their request for an explanation of why the examiner believes that undue experimentation is required to practice the claimed invention in the absence of a deposit.

The examiner also has objected to the specification on the basis that the application does "not enable the production of all transgenic mammals which produce heterologous protein C in their milk." Office action at page 3. Applicants respectfully submit that the present amendments overcome this basis for objection.

To expedite prosecution, applicants have amended the independent claims to state that transgenic animals are selected from the group consisting of mice, rats, rabbits, pigs, sheep, goats and cows. Applicants have demonstrated the successful production of transgenic mice and transgenic pigs that carry the DNA constructs of the present invention. In addition, applicants note that those of skill in the art **routinely use** rabbits, pigs,

sheep, goats and cows to produce foreign proteins in milk. See Rudolph, "Regulatory Issues Relating to Protein Production in Transgenic Animal Milk," *Genetic Engineering News 15*: 16 (July 1995), which is attached to this response as Exhibit 2. In fact, the ability to produce transgenic sheep and goats that secrete human proteins in milk was within the skill of the art at least four years ago. See the first paragraph of Rudolph, "Advances Continue in Production of Proteins in Transgenic Animal Milk," *Genetic Engineering News 15*: 8 (October 15, 1995), which is attached to this response as Exhibit 3. Thus, the Markush group of the amended claims is supported by data that are part of this record and by evidence of the level of skill in the art.[1]

With regard to the issue of sufficient support for the production of transgenic animals, applicants respectfully direct the examiner's attention to Clark *et al.*, U.S. patent No. 5,322,775 (1994), which is attached to this response as Exhibit 4. The claims of the '775 patent relate to the production of a "transgenic, non-human placental mammal," a class that encompasses many more species than the Markush group of the presently amended claims. At the same time, the '775 patent discloses the production of only two species of transgenic mammals using technology that *predates by three years* the state of the art at the time that the parent case of the present application had been filed. Especially in view of the '775 patent, applicants must be deemed to have provided adequate support for their new Markush group.

Finally, applicants understand that the examiner has objected to the specification on the basis that there is a lack of support for the scope of the language "DNA sequence comprising substantially the 5' 4.2 kb *Sau*3A - *Kpn*1 promoter of the mouse

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[1] Applicants appreciate the examiner's concerns regarding posttranslational modification of foreign proteins in various animal species. Regardless of such theoretical concerns, however, those of skill in the art <u>are producing</u> transgenic animals that synthesize functional foreign proteins.

whey acidic protein gene, or a variant thereof" and "a DNA sequence encoding a polypeptide having protein C activity." To expedite prosecution, applicants have amended the claims by focusing on a DNA sequence comprising the 5' 4.2 kb *Sau*3A - *Kpn*1 promoter of the mouse whey acidic protein gene and a DNA sequence encoding protein C, as described below. Thus, this basis for objection now is moot.[2]

In light of the amendments and remarks above, applicants respectfully request the examiner to withdraw the objection to the specification under the first paragraph of 35 USC §112.

II. Rejection Under 35 USC §112, First Paragraph

The examiner has rejected claims 1-15 under 35 USC §112, first paragraph, for the reasons set forth in the objection to the specification.

Having overcome this basis for objection to the specification, applicants submit that the rejection to the claims may be properly withdrawn.

In light of the amendments and remarks above, applicants request the examiner to withdraw the rejection of the claims under 35 USC §112, first paragraph. Reconsideration of the claims is respectfully requested.

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[2] Applicants note that the '775 patent and Clark *et al.*, U.S. patent No. 5,476,995 (1995) [Exhibit 5] contain claims that relate to the use of "a β-lactoglobulin promoter," while the specifications disclose the isolation of a single promoter from sheep. In contrast, applicants' amended claims are focused on the use of a particular murine WAP promoter that is described in the present specification. If the broad class of β-lactoglobulin promoters is supported by the specifications of the '775 and '995 patents, then the present specification provides more than adequate support for the murine WAP promoter.

III. Rejections Under 35 USC §112, Second Paragraph

The examiner has rejected claims 1-15 under 35 USC §112, second paragraph, as being indefinite for failing to particularly point out and distinctly claim the subject matter which the applicants regard as the invention. In particular, the examiner has objected to the language "comprises substantially" or variations thereof in claims 1, 3, 6, 11, 12 and 14. In addition, the examiner has rejected claim 3 on the basis that the language "variants thereof" renders the claim indefinite. Applicants respectfully submit that the present amendments overcome this basis for rejection.

To expedite prosecution, applicants have deleted the term "substantially" from the description of the WAP promoter in claims 1, 3, 6, 11, 12 and 14. Moreover, applicants have amended claim 3 by deleting the language "variants thereof" from the description of the WAP promoter.[3] Thus, this basis for rejection of the claims now is moot.

In light of the amendments and remarks above, applicants request the examiner to withdraw the rejection to the claims under 35 USC §112, second paragraph. Reconsideration of the claims is respectfully requested.

IV. First Rejection Under 35 USC §103

The examiner has rejected claims 1-10 and 12-15 under 35 USC §103, as being unpatentable over Pittius *et al., Proc. Nat'l Acad. Sci. USA 85*: 5874 (1988), in view of Grinnell *et al., Bio/Technology 5*: 1189 (1987), Brinster *et al., Proc. Nat'l Acad.*

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[3] Applicants respectfully assert, however, that the scope of the amended claims includes the use of a DNA sequence comprising the 5' 4.2 kb *Sau*3A - *Kpn*I promoter of the mouse WAP gene and any "insubstantial" variation of the promoter, according to the test for insubstantial difference provided by the Federal Circuit in *Hilton Davis Chemical Co. v. Warner-Jenkinson Co.*, 35 USPQ2d 1641 (Fed. Cir. 1995).

Sci. USA 85: 836 (1988), Campbell *et al.*, *Nucl. Acids Res. 12*: 8686 (1984), and Clark *et al.*, *TIBTECH 5*: 20 (1987), for reasons of record. Applicants respectfully traverse this basis for rejection.

Applicants believe that they have clearly explained their position regarding the lack of a *prima facie* case of obviousness in their previous responses. Nevertheless, applicants respectfully request the examiner to reconsider the relevance of the Pittius publication which is the primary reference.

In their studies, Pittius *et al.* used a WAP promoter of about 2.6 kilobases. See page 5874, second column, second full paragraph of the Pittius publication. The examiner's position is that:

> [Applicants'] 4.2kb *Sau*3A - *Kpn*I fragment of the whey acidic promoter is obvious over the smaller fragment taught by Pittius, as the optimization of expression would be within the scope of skills of the ordinary artisan.

Office action of July 22, 1993, at page 11. Applicants respectfully submit that the examiner's argument would be relevant if the present claims related to "a WAP promoter that is an optimized promoter of the 2.6 kb promoter of Pittius *et al.*"

Yet applicants' claims are not so broad. The present claims relate to a DNA sequence comprising the 5' 4.2 kb *Sau*3A - *Kpn*1 promoter of the mouse WAP gene. The examiner has not presented prior art that suggests the modification of the Pittius promoter to obtain the promoter recited in applicants' claims. This deficiency of the primary reference is not corrected by Grinnell's description of the expression of protein C by tissue culture cells, by Brinster's suggestion that intron sequences enhance transgene expression in mice, by Campbell's disclosure of a murine WAP gene, or by Clark's suggestion of the desirability of producing foreign proteins in the milk of transgenic animals. Since the secondary references cannot cure the deficiency of the Pittius publication, the combination of the

cited references cannot support a *prima facie* case of obviousness.

Moreover, even if the cited references seemed to render a *prima facie* case of obviousness, the nonobviousness of the claimed invention is evidenced by the surprising results obtained with applicants' promoter. In particular, the 4.2 kb WAP promoter provides an ***unexpected*** degree of tissue specificity and improved gene expression, as shown by studies with the human protein C gene. See pages 12-14 of the response filed on April 21, 1995, and see Example 11 of the present specification.

In addition, applicants present new data provided by Harry Meade, Research Director of Genzyme Transgenics Corporation, from studies of transgenic mice that carried an α_1-antitrypsin gene under the control of the 4.2 kb WAP promoter or under the control of a 2.3 kb WAP promoter. As shown in the attached table [Exhibit 6], the majority of transgenic mouse lines carrying the small promoter produced less than 1 mg/ml of the foreign protein. In fact, three of five lines produced less than 0.1 mg/ml. In contrast, five of eight lines that carry the 4.2 kb WAP promoter produced greater than 1 mg/ml of the foreign protein, while three lines produced at least 50 mg/ml. Thus, the 4.2 kb WAP promoter unexpectedly stimulates the expression of a second foreign gene, α_1-antitrypsin, in a transgenic animal.

In sum, the surprising stimulation of either protein C gene expression or α_1-antitrypsin gene expression by the 4.2 kb WAP promoter provides evidence for the nonobviousness of the claimed invention. For this reason alone, the rejection under §103 should be withdrawn.

In light of the remarks above, applicants request the examiner to withdraw the rejection to the claims under 35 USC §103. Reconsideration of the claims is respectfully requested.

V. Second Rejection Under 35 USC §103

The examiner has rejected claim 11 under 35 USC §103, as being unpatentable over Colpan *et al.*, *J. Chromatography 296*: 339 (1984), in view of Hogan *et al.*, MANIPULATION OF THE MOUSE EMBRYO, pages 153-203 (Cold Spring Harbor Press 1986), for reasons of record. Applicants respectfully traverse this basis for rejection.

Applicants understand the examiner's position to be (1) that Colpan teaches the purification of plasmid DNA by anion exchange HPLC, (2) that Hogan teaches that DNA used in the production of transgenic animals should be free of impurities, and therefore, (3) that:

> the ordinary artisan with the teachings of the prior art would have been offered a reasonable expectation of success in the production of the transgenic non-human mammal as claimed when the DNA construct comprising the transgene had been purified by HPLC.

Office action of July 22, 1993, at page 12.

Applicants note, however, that claim 11 is not directed to a general method for producing transgenic animals that comprises purification of any DNA molecule by HPLC. Rather, claim 11 is directed to a method for producing transgenic animals that requires the purification of particular, recited DNA molecules. That is, claim 11 *requires* the use of a DNA molecule comprising the 4.2 kb WAP promoter, a DNA molecule comprising a fragment of the human protein C gene, or a DNA molecule comprising the 4.2 kb WAP promoter linked to a fragment of the human protein C gene. Neither the Colpan reference nor the Hogan publication suggests the production of transgenic animals that carry these DNA molecules. At least for this reason, therefore, the combination of the cited references fails to suggest the invention of claim 11.

In light of the remarks above, applicants request the examiner to withdraw the rejection to claim 11 under 35 USC §103. Reconsideration of the claim is respectfully requested.

CONCLUSION

Applicants request reconsideration of the claims on their merits and respectfully solicit early notification of an allowance. If Examiner Crouch should have any questions or believes a telephone discussion would expedite prosecution, the examiner is invited to contact the undersigned at the telephone number listed below.

Respectfully submitted,

January 29, 1996
Date

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